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Human leukocyte antigen alleles and the response to pegylated interferon/ribavirin therapy in chronic hepatitis C patients

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ABSTRACT

Human leukocyte antigens (HLAs) may play a role in the clinical evolution of hepatitis C virus (HCV) infection. The present study was aimed at elucidating the association between the HLA loci and responses to combination therapy with pegylated interferon-alpha 2a (PEG-IFN) and ribavirin in Taiwanese. We enrolled a total of 208 treatment-naïve Taiwanese chronic hepatitis C (CHC) patients treated with combination therapy. Patients with sustained virological response (SVR) had a significantly higher frequency of genotype non-1b infection, lower pretreatment HCV RNA levels and a higher frequency of mild hepatic fibrosis (fibrosis score: F: 0–2). The HLA A24 and B40 alleles were significantly associated with SVR after adjusted for the other three confounding factors including HCV genotype, hepatic fibrosis and pretreatment serum HCV RNA levels. Haplotypes (B40-DRB1*3, B46- DRB1*9, Cw1- DQB1*3, and Cw1- DRB1*9) were significantly associated with SVR to combination therapy. For 167 patients with genotype 1b infection and viral load < or =5.6 log IU/ml or genotype non-1b infection, the B46 was significantly associated with sustained response with OR (odds ratio) [95% CI (confidence interval) of 0.047 (0.168–0.988)]. Haplotypes B40-DRB1*3, B46- DRB1*9, Cw1- DQB1*3, Cw1- DRB1*9 and DQB1*3- DRB1*9 were found to be associated with SVR to PEG-IFN/ribavirin therapy with OR (95% CI) of 0.179 (0.032–0.989), 0.313 (0.107–0.918), 0.350 (0.145–0.845), 0.282 (0.105–0.759) and 0.412 (0.174–0.978), respectively. We concluded that the virological and the host immunogenetic factors may possibly predict the response to combination therapy in CHC patients.

1. Introduction

Combination therapy with pegylated interferon-alpha (PEG-IFN) and ribavirin has been recommended as standard therapy for patients with chronic hepatitis C virus (HCV) infection (NIH Consens State Sci Statements, 2002). Several clinical factors have been indicated as predictors of the response to combination therapy. The viral genotype, one of the most important viral predictors, has become the critical determinant of the length of combination therapy (NIH Consens State Sci Statements, 2002; Strader et al., 2004). On the other hand, some host factors such as age, gender, race, insulin resistance and host immune responses may also significantly affect drug response (Alberti and Benvegnù, 2003; Dai et al., 2009). The role of immunogenetic factors has been clearly delineated in several chronic viral infections of humans, including chronic hepatitis C (CHC) and chronic hepatitis B (CHB) (Thomas and Thursz, 1997; Zavaglia et al., 1996). Since PEG-IFN has direct antiviral effects and ribavirin promotes a type 1 cytokine-mediated immune response that can enhance antiviral immune responses (Morishima et al., 2006; Tam et al., 1999), there might be an association of immunogenetic characteristics and response to anti-viral therapy.

The human leukocyte antigens (HLAs), encoded by the major histocompatibility complexes (MHC), may play a role in the host response to infection (Kaslow et al., 1996; Roger, 1998). With the association with presenting antigen to $CD8^+$ cytotoxic T cells and

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$CD4^+$ helper T cells, HLA Class I and class II molecules are central to the host immune response and are thus ideal candidate genes to be investigated for associations with HCV outcomes (Koziel, 1997). Specific HLA alleles might be related to persisted or spontaneous clearance of HCV (Alric et al., 1997; Fanning et al., 2001; Lechmann et al., 1999; Thio et al., 2002), HCV viral load (Fanning et al., 2001) and progression of liver fibrosis (Höhler et al., 1997) or the antiviral response to conventional IFN monotherapy therapy (Kikuchi et al., 1998; Sim et al., 1998). Only few reports showed the association between HLA alleles and response to standard or conventional-IFN/ribavirin therapy (Jiao and Wang, 2005; Romero-Gómez et al., 2003). To our knowledge, however, no study was carried out to investigate the impact of HLA alleles on the response to combination therapy with PEG-IFN and ribavirin.

Taiwan is a hyperendemic country for CHB and several HCV hyperendemic townships have been discovered with the anti-HCV prevalence more than 6–30% in southern Taiwan (Chuang et al., 2006; Dai et al., 2008). Favorable rates of sustained virologic response (SVR) have been reported among Taiwanese naïve CHC patients after combination therapy with IFN/ribavirin (Yu et al., 2007, 2008). We have previously conducted studies trying to develop genetic models and investigating the polymorphisms of the cytokine genes for predicting the response to anti-HCV therapy in Taiwan (Dai et al., 2005, 2006). Our previous report has shown that some HLA alleles might be associated with the response to IFN monotherapy in Taiwanese CHC patients (Yu et al., 2003). With the lack of investigation in the impact of the gene polymorphisms and heterozygosity at HLA classes I and II alleles on the response to combination therapy with PEG-IFN and ribavirin, we conducted the present study aiming to elucidate the association between the HLA loci and responses to PEG-IFN/ribavirin therapy in Taiwanese. The interactive association between specific HLA haplotypes and therapeutic response were also investigated after adjustment for the potential confounders.

2. Materials and methods

2.1. Patients

A total of 208 Taiwanese treatment-naïve CHC patients were enrolled in the present study. All were positive for HCV antibodies (anti-HCV, third-generation, Abbott, North Chicago, IL) and serum HCV RNA. Patients were negative for hepatitis B surface antigen (HBsAg, Abbott, North Chicago, IL), without human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson's disease, Alpha 1-antitrypsin deficiency, decompensated cirrhosis, overt hepatic failure, current or past history of alcohol abuse, psychiatric condition, previous liver transplantation, or evidence of hepatocellular carcinoma. All patients had a liver biopsy showing chronic hepatitis of different severity within 6 months of starting PEG-IFN/ribavirin treatment. Liver histology was graded and staged according to the scoring system described by Knodell and Scheuer, (Knodell et al., 1981; Scheuer, 1991) by a single pathologist who was blinded to the treatment of each patient. The study complied with the Declaration of Helsinki, was approved by the ethics committee of Kaohsiung Medical University Hospital and patients had given their informed consent.

2.2. Detection/genotyping/quantification of serum HCV RNA

Serum HCV RNA at the end of treatment and 24 weeks after therapy was determined by standardized automated qualitative polymerase chain reaction (PCR) (Cobas Amplicor Hepatitis C Virus Test, version 2.0; Roche Diagnostics, Branchburg, NJ; detection limit: 50 IU/ml). HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers described by Okamoto et al. (1993). Serum HCV RNA levels before combination therapy were measured using the branched DNA assay (Versant HCV RNA 3.0, Bayer, Tarrytown, NJ; the quantification range: 615–7,690,000 IU/ml).

2.3. Combination therapy with PEG-IFN 2a and ribavirin

All patients were treated with recombinant PEG-IFN 2a (PEGASYS®, Hoffmann-La Roche) at a dose of 180 μg once a week subcutaneously and ribavirin daily in two divided doses by mouth for 24 weeks. The dose of ribavirin was based on body weight (1000 mg ribavirin for weight ≤75 kg and 1200 mg ribavirin for weight >75 kg). The presence of HCV RNA in the serum was assessed every 3 months. Patients achieving SVR in terms of clearance of serum HCV RNA by RT-PCR at the end of the therapy and for 6 months after the cessation of therapy were grouped as sustained responders. All other patients did not achieve SVR were classified as non-responders.

2.4. HLA typing

Genomic DNA was purified from whole blood by the QIAamp blood kit (Qiagen, Valencia, CA), and the quantity and purity DNA sample to be used for PCR should be re-suspended in sterile water or in 10 mM Tris–HCl, pH 8.0–9.0 at an optimal concentration of 20 ng/μl with the A260/A280 ratio of 1.65–1.80. The HLA class I and II alleles were determined using low-resolution PCR/sequence-specific oligonucleotide probes DNA-based typing method (LABType® SSO One Lambda Inc.) according to the Manufacturer's instructions. LABType® applies Luminex technology to the reverse SSO DNA typing method. First, target DNA was PCR-amplified using a group-specific primer. The PCR product was biotinylated, which allows it to be detected using R-phycoerythrin-conjugated streptavidin (SAPE). The PCR product was denatured and allowed to rehybridize to complementary DNA probes conjugated to fluorescently coded microspheres. A flow analyzer, the LABScan™ 100, identified the fluorescent intensity of PE (phycoerythrin) on each microsphere. The assignment of the HLA typing was based on the reaction pattern compared to patterns associated with published HLA gene sequences.

2.5. Statistical analysis

The population frequency of alleles (allelic carriage frequency) was calculated as follows: frequency of the allele in position 1 plus frequency of the allele in position 2, without double counting of homozygotes, divided by the number of subjects. We further designed allelic variables as continuous dependents (null = 0, heterozygosity = 1, and homozygosity = 2) to analyze the gene-dosage effect of the virologic responses with PEG-IFN plus ribavirin combination therapy by using logistic regression model after controlling confounders. Frequency was compared between groups using the chi-square test with Yates' correction or Fisher's exact test. For all tests a P value <0.05 was considered to be significant, and significant P values were corrected (Bonferroni correction) for the number of alleles detected at each locus. Odds ratios (ORs) were calculated for different alleles, whenever a significant P value in the distribution of a specific allele was observed. Group means were compared using the Student's t-test. Serum HCV RNA levels were expressed as the mean ± standard deviation after logarithmic transformation of original values. Linkage disequilibrium was analyzed by online software (Genepop; http://wbiomed.curtin.edu.au/genepop). Multivariate logistic regression was used to analyze demographic, virologic and histologic factors associated with response to com-

Table 1
Factors associated with response to pegylated interferon-alpha 2a and ribavirin in 208 chronic hepatitis C patients.

Factors	Non-responder ($N=59$)	Sustained responder ($N=149$)	P
Age (year)	51.1 ± 11.1	50.2 ± 11.4	0.607
Gender			0.585
Male, *n* (%)	32 (54.2)	87 (58.4)	
Female, *n* (%)	27 (45.8)	62 (41.6)	
Pretreatment AST[a] levels (IU/L)	102.1 ± 54.8	111.1 ± 80.0	0.431
Pretreatment ALT[b] levels (IU/L)	143.2 ± 108.5	177.2 ± 154.8	0.125
Pretreatment HCV RNA levels (log IU/ml)	5.57 ± 0.83	4.96 ± 1.14	**<0.001**
HCV genotype			**<0.001**
1b, *n* (%)	43 (72.9)	37 (24.8)	
Non-1b, *n* (%)	16 (27.1)	112 (75.2)	
Hepatic fibrosis score			**0.001**
F: 0–2, *n* (%)	36 (61.0)	123 (82.6)	
F: 3–4, *n* (%)	23 (39.0)	26 (17.4)	

[a] AST: aspartate aminotransferase.
[b] ALT: alanine aminotransferase.

bination treatment in CHC patients. The adjusted OR and 95% confidence interval (CI) of each possible significant HLA allele (P value below 0.1 in univariate analysis) was obtained by using a logistic regression model to control the virological and histologic confounders (pretreatment serum HCV RNA levels, HCV genotype and cirrhosis). All procedures were performed by using the package SPSS statistical software (version 12.0; SPSS Inc. Chicago, IL).

3. Results

3.1. Response to combination therapy with PEG-IFN 2a and ribavirin

Of the 208 CHC patients (male/female: 119/89, mean age: 50.4 ± 11.3 years), the mean AST, ALT and pretreatment HCV RNA level were 108.5 ± 73.7 IU/L, 167.5 ± 143.7 IU/L and 5.13 ± 1.21 log IU/ml, respectively. Eighty (38.5%) patients were infected by HCV genotype 1b and 49 (23.6%) patients had severe fibrosis (fibrosis score: F: 3–4). After PEG-IFN/ribavirin therapy for 24 weeks, 71.6% (149/208) of patients achieved SVR. Comparison of the clinical factors between sustained responders and non-responders in univariate analyses is shown in Table 1. Sustained responders had a significantly higher frequency of genotype non-1b infection, lower pretreatment HCV RNA levels and a higher frequency of mild hepatic fibrosis (fibrosis score: F: 0–2) (P <0.001, <0.001 and <0.01, respectively). Based on multivariate regression analyses, the significant factors associated with responses to combination therapy were HCV genotype [1b vs non-1b, odds ratio (OR)/95% confidence interval (CI): 0.125/0.060–0.261], hepatic fibrosis (F: 3–4 vs F: 0–2, OR/95% CI: 0.345/0.149–0.800) and pretreatment serum HCV RNA levels (per 1 log IU/ml increase, OR/95% CI: 0.559/0.385–0.813).

3.2. HLA class I and II alleles in sustained responders and non-responders

The allele frequencies of HLA-A, -B, -C, -DR and -DQ in the sustained responders and non-responders were compared (Tables 2 and 3). In univariate analysis the frequency of B40 was significantly lower in sustained responders than in non-responders

Table 2
HLA- A, B, C alleles and response to pegylated interferon-alpha 2a and ribavirin in chronic hepatitis C patients.

Allele	No.	Non-responder *n* (%), ($N=59$)	Sustained responder *n* (%), ($N=149$)	Univariate analysis			Multivariate analysis[a] (allele possessing effect[b])			Multivariate analysis[a] (allele gene-dosage effect[c])		
				OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
A02	108	32 (54.2)	76 (51.0)	0.878	0.480–1.608	0.674	0.627	0.300–1.313	0.216	0.686	0.374–1.258	0.223
A11	106	29 (49.2)	77 (51.7)	1.106	0.605–2.022	0.743	0.908	0.442–1.866	0.793	1.002	0.568–1.768	0.994
A24	72	16 (27.1)	56 (37.6)	1.618	0.834–3.140	0.153	2.289	1.017–5.150	**0.045**	2.166	1.060–4.425	**0.034**
A33	60	19 (32.2)	41 (27.5)	0.799	0.416–1.537	0.501	0.758	0.350–1.643	0.483	0.909	0.456–1.814	0.788
B15	40	7 (11.9)	33 (22.1)	2.113	0.878–5.089	0.090	1.731	0.637–4.707	0.282	1.577	0.684–3.633	0.285
B27	22	6 (10.2)	16 (10.7)	1.063	0.395–2.862	0.904	1.472	0.465–4.661	0.511	1.121	0.397–3.163	0.829
B40	86	31 (52.5)	55 (36.9)	0.528	0.287–0.972	**0.039**[#]	0.475	0.229–0.985	**0.045**	0.599	0.327–1.097	0.097
B46	43	14 (23.7)	29 (19.4)	0.777	0.377–1.602	0.493	0.643	0.267–1.549	0.325	0.650	0.272–1.553	0.333
B54	14	4 (6.8)	10 (6.7)	0.989	0.298–3.287	1.000	1.963	0.489–7.881	0.342	2.312	0.686–7.79	0.176
B56	10	4 (6.8)	6 (4.0)	0.577	0.157–2.123	0.474	0.271	0.059–1.245	0.093			
B58	43	9 (15.3)	34 (22.8)	1.643	0.733–3.678	0.225	1.085	0.420–2.803	0.865	1.280	0.531–3.086	0.582
CW01	68	20 (34)	48 (32.2)	0.927	0.489–1.756	0.816	0.941	0.433–2.047	0.879	0.909	0.436–1.895	0.798
CW03	110	30 (50.1)	80 (53.7)	1.121	0.613–2.049	0.711	0.525	0.350–0.788	0.869	0.926	0.524–1.635	0.790
CW04	22	6 (10.2)	16 (10.7)	1.063	0.395–2.862	0.904	1.090	0.345–3.448	0.883	0.926	0.333–2.574	0.883
CW07	80	23 (39.0)	57 (38.3)	0.970	0.522–1.800	0.923	0.825	0.394–1.726	0.610	0.643	0.364–1.136	0.128
CW08	34	6 (10.2)	28 (18.8)	2.011	0.799–5.227	0.130	2.805	0.945–8.326	0.063	2.193	0.832–5.780	0.112
CW14	10	3 (5.1)	7 (4.7)	0.920	0.230–3.685	1.000	1.465	0.298–7.203	0.639	1.579	0.376–6.631	0.533
CW15	15	4 (6.8)	11 (7.4)	1.096	0.335–3.589	1.000	1.919	0.468–7.874	0.365	1.930	0.489–7.62	0.348

Note: OR: odds ratio; CI: confidence interval.
[a] Adjusted by pretreatment HCV genotype, viral load and liver fibrosis.
[b] HLA allele homozygote or heterozygote = 1; null = 0.
[c] HLA allele homozygote = 2; heterozygote = 1; and null = 0.
[#] Bonferroni correction: not statistically significant.

Table 3
HLA DQB1 and DRB1 alleles and response to pegylated interferon-alpha 2a and ribavirin in chronic hepatitis C patients.

Allele	No	Non-responder *n* (%), (*N* = 59)	Sustained responder *n* (%), (*N* = 149)	Univariate analysis			Multivariate analysis[a] (allele possessing effect[b])			Multivariate analysis[a] (allele gene-dosage effect[c])		
				OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
DQB1*02	26	6 (10.2)	20 (13.4)	1.370	0.521–3.601	0.522	1.066	0.337–3.369	0.914	1.427	0.539–3.778	0.474
DQB1*03	130	41 (69.5)	89 (59.7)	0.662	0.348–1.262	0.209	0.749	0.356–1.603	0.456	0.871	0.558–1.359	0.543
DQB1*04	39	11 (18.6)	28 (18.8)	1.010	0.466–2.188	0.980	0.806	0.319–2.031	0.647	0.818	0.344–1.947	0.649
DQB1*05	73	19 (32.2)	54 (36.2)	1.197	0.631–2.270	0.582	1.345	0.621–2.909	0.542	1.213	0.651–2.262	0.542
DQB1*06	65	17 (28.8)	48 (32.2)	1.174	0.607–2.272	0.633	0.935	0.426–2.052	0.867	1.025	0.531–1.977	0.942
DRB1*01	2	0 (0.0)	2 (1.3)	0.714	0.654–0.778	1.000						
DRB1*03	31	10 (16.9)	21 (14.1)	0.804	0.352–1.829	0.602	0.693	0.263–1.831	0.460	0.853	0.334–2.175	0.739
DRB1*04	68	16 (27.1)	52 (34.9)	1.441	0.741–2.803	0.281	1.850	0.835–4.102	0.130	1.344	0.68–2.655	0.395
DRB1*07	11	3 (5.1)	8 (5.4)	1.059	0.271–4.137	1.000	1.220	0.200–7.447	0.829			
DRB1*08	35	10 (16.9)	25 (16.8)	0.988	0.442–2.208	0.976	0.996	0.381–2.607	0.994			
DRB1*09	50	18 (30.5)	32 (21.5)	0.623	0.316–1.228	0.169	0.583	0.257–1.324	0.197	0.596	0.267–1.333	0.207
DRB1*10	13	4 (6.8)	9 (6.0)	0.884	0.261–2.989	1.000	0.986	0.206–4.709	0.986			
DRB1*11	18	5 (8.5)	13 (8.7)	1.032	0.351–3.035	0.954	0.633	0.178–2.258	0.481	0.660	0.194–2.249	0.507
DRB1*12	49	17 (28.8)	32 (21.5)	0.676	0.340–1.342	0.261	0.867	0.388–1.938	0.729	0.901	0.437–1.86	0.778
DRB1*13	13	2 (3.4)	11 (7.4)	2.272	0.488–10.575	0.358	1.084	0.198–5.942	0.926			
DRB1*14	36	8 (13.6)	28 (18.8)	1.475	0.630–3.455	0.369	1.806	0.654–4.992	0.254			
DRB1*15	54	14 (23.7)	40 (26.8)	1.180	0.585–2.377	0.644	1.124	0.490–2.583	0.782			
DRB1*16	22	7 (11.9)	15 (10.1)	0.832	0.321–2.156	0.704	0.821	0.254–2.659	0.743			

Note: OR: odds ratio; CI: confidence interval.
[a] Adjusted by pretreatment HCV genotype, viral load and liver fibrosis.
[b] HLA allele homozygote or heterozygote = 1; null = 0.
[c] HLA allele homozygote = 2; heterozygote = 1; and null = 0.

(*P* = 0.039), but it did not achieve statistical significance when the Bonferroni correction was applied. The OR and 95% CI of each possible significant HLA allele (*P* value below 0.1 in univariate analysis) was further adjusted by the factors including pretreatment HCV genotype (1b vs non-1b), HCV viral load, and liver fibrosis (mild: F: 0–2 vs advanced: F: 3–4) using a logistic regression model to control the virological and histologic confounders (Table 2). The A24 and B40 was significantly associated with response with the OR (95% CI) of 2.289 (1.017–5.150) and 0.475 (0.229–0.985), respectively. The nature and extent of homozygote HLA genotype was examined to investigate the gene-dosage effect of HLA alleles on the response to PEG-IFN/ribavirin therapy (Table 2). Ninety-three of 136 (68.4%) patients without A24, 50 of 65 (76.9%) patients with one copy of A24 and six of 7 (85.7%) carrying two copies of A24 allele were sustained responders. The gene-dosage correlation to response to combination therapy was observed in A24 allele with adjusted OR (95% CI) of 2.166 (1.060–4.425). A possible association between specific HLA allele combinations and sustained response to PEG-IFN/ribavirin therapy was evaluated after adjustment with virological and histological confounders. After analyzing the relationship between sustained response and all haplotypes in linkage disequilibrium in univariate analysis, the frequency of B40-DRB1*3 was significantly lower in sustained responders than in non-responders (*P* = 0.043), but it did not achieve statistical significance after Bonferroni correction (Table 4). By using the logistic regression model to control the virological and histologic confounders, we found four different haplotypes were significantly associated with sustained response to PEG-IFN/ribavirin therapy: B40-DRB1*3, B46- DRB1*9, Cw1-DQB1*3, and Cw1- DRB1*9 with OR (95% CI) of 0.170 (0.030–0.963), 0.304 (0.093–0.996), 0.429 (0.187–0.983) and 0.316 (0.107–0.937), respectively.

3.3. Viral factors versus HLA class I and II alleles in sustained responders and non-responders

The associations between SVR to PEG-IFN/ribavirin therapy and HLA alleles were evaluated in different groups of CHC patients stratified by the pretreatment virological factors: HCV genotypes and viral loads. Patient with genotype 1b infection and with high viral load (>5.6 log IU/ml) were grouped as high-risk group (group A) and the other patients were grouped as non-high-risk group (group B) (genotype 1b infection with viral load < or =5.6 log IU/ml or genotype non-1b infection) according to our previous prospective study (Yu et al., 2004). For 167 group B patients, presence of advanced fibrosis (F: 3–4) was the significant factor associated with response to PEG-IFN/ribavirin therapy in univariate analysis (Table 5). The different SVR rates in patients with mild and advanced fibrosis were not observed in group A patients. Further analysis by using multiple logistic regression, the advanced fibrosis was significantly associated with SVR (OR/95% CI: 0.305/0.128–0.726). The frequencies of B40 and DQB1*3 were significantly lower in the sustained responders than in the non-responders, however all the associations did not achieve statistical significance when the Bonferroni correction was applied. The OR and 95% CI of each possible significant HLA allele (*P* value below 0.1 in univariate analysis) was further adjusted by liver fibrosis (mild vs advanced) using logistic regression model to control the histologic confounders (Table 5). The B46 was significantly associated with sustained response with OR (95% CI) of 0.047 (0.168–0.988). Additionally, five different haplotypes, B40-DRB1*3, B46- DRB1*9, Cw1- DQB1*3, Cw1- DRB1*9 and DQB1*3- DRB1*9, were found to be associated with SVR to PEG-IFN/ribavirin therapy in group B patients with OR (95% CI) of 0.179 (0.032–0.989), 0.313 (0.107–0.918), 0.350 (0.145–0.845), 0.282 (0.105–0.759) and 0.412 (0.174–0.978), respectively.

Table 4
Estimate of haplotype frequency in linkage disequilibrium HLA alleles and response to pegylated interferon-alpha 2a and ribavirin in chronic hepatitis C patients.

Allele	No.	Non-responder *n* (%), (*N* = 59)	Sustained responder *n* (%), (*N* = 149)	Univariate analysis			Multivariate analysis[a]		
				OR	95% CI	*P*	OR	95% CI	*P*
A11-B27	16	3 (5.1)	13 (8.7)	1.784	0.490–6.504	0.565	2.492	0.558–11.125	0.232
A11-Cw3	48	16 (27.1)	32 (21.5)	0.735	0.367–1.472	0.384	0.491	0.209–1.151	0.102
A11-Cw12	18	3 (5.1)	15 (10.1)	2.090	0.582–7.502	0.249	2.251	0.466–10.865	0.313
A24-B40	32	8 (13.6)	24 (16.1)	1.224	0.516–2.904	0.646	1.484	0.528–4.173	0.454
A24-B54	9	1 (1.7)	8 (5.4)	3.291	0.402–26.906	0.450	7.376	0.726–74.986	0.091
A24-DRB1*04	32	7 (11.9)	25 (16.8)	1.498	0.610–3.678	0.376	2.353	0.812–6.821	0.115
A33-B46	7	3 (5.1)	4 (2.7)	0.515	0.112–2.374	0.387	0.369	0.060–2.273	0.282
A33-DQB1*2	15	2 (3.4)	13 (8.7)	2.724	0.596–12.462	0.241	2.597	0.453–14.879	0.284
A33-DQB1*3	31	13 (22.0)	18 (12.1)	0.486	0.221–1.070	0.069	0.618	0.242–1.539	0.301
B40-Cw1	20	7 (5.1)	13 (8.7)	0.710	0.267–1.878	0.489	1.013	0.318–3.230	0.983
B40-Cw7	42	14 (23.7)	28 (18.8)	0.744	0.359–1.539	0.424	0.608	0.041–1.207	0.258
B40-DQB1*2	8	4 (6.8)	4 (2.7)	0.379	0.092–1.570	0.227	0.224	0.041–1.207	0.082
B40-DRB1*3	8	5 (8.5)	3 (2.0)	0.222	0.051–0.960	**0.043**[#]	0.170	0.030–0.963	**0.045**
B40-DRB1*4	33	8 (13.6)	25 (16.8)	1.285	0.544–3.038	0.676	1.218	0.451–3.284	0.697
B46-Cw1	32	10 (16.9)	22 (14.8)	0.849	0.375–1.921	0.694	1.004	0.365–2.760	0.994
B46-DRB1*9	22	9 (15.3)	13 (8.7)	0.531	0.214–1.319	0.168	0.304	0.093–0.996	**0.041**
Cw1-DQB1*3	51	19 (32.2)	32 (21.5)	0.576	0.294–1.127	0.105	0.429	0.187–0.983	**0.046**
Cw1-DRB1*9	27	11 (18.6)	16 (10.7)	0.525	0.228–1.211	0.126	0.316	0.107–0.937	**0.037**
Cw3-DQB1*3	60	20 (33.9)	40 (26.8)	0.716	0.374–1.37	0.312	0.589	0.268–1.293	0.187
Cw7-DRB1*8	17	7 (11.9)	10 (6.7)	0.534	0.193–1.478	0.262	0.303	0.088–1.035	0.057
DQB1*3-DRB1*9	49	18 (30.5)	31 (20.1)	0.598	0.303–1.182	0.137	0.538	0.235–1.232	0.143
DQB1*3-DRB1*14	13	1 (1.7)	12 (8.1)	5.080	0.646–39.978	0.116	7.334	0.722–74.455	0.092
DQB1*5-DRB1*3	6	3 (5.1)	3 (2.0)	0.384	0.075–1.957	0.355	0.197	0.300–1.298	0.091
DQB1*6-DRB1*4	17	3 (5.1)	14 (9.4)	1.936	0.535–6.999	0.406	2.955	0.642–13.601	0.164

Note: OR: odds ratio; CI: confidence interval.
[a] Adjusted by pretreatment HCV genotype, viral load and liver fibrosis.
[#] Bonferroni correction: not statistically significant.

4. Discussion

In the present study we investigated the possible association between HLA class I and II alleles and response to PEG-IFN/ribavirin therapy in patients with CHC. By comparing the frequencies of HLA alleles and haplotypes in linkage disequilibrium between patients who responded and those who did not respond to combination treatment, associations between SVR and HLA A24 allele, between non-response and B40 allele, B40-DRB1*3, B46- DRB1*9, Cw1-DQB1*3, and Cw1- DRB1*9 haplotypes, and a gene-dosage effect of A24 allele on the therapeutic response were suggested. All the associations lost significance after the results were corrected for the number of alleles tested. The Bonferroni correction that was applied has been considered too stringent for this analysis (Perneger, 1998). The A24 allele showed a significant association with SVR and the B40 allele and haplotypes including B40-DRB1*3, B46- DRB1*9, Cw1- DQB1*3, Cw1- DRB1*9 and DQB1*3- DRB1*9 maintained a significant association with non-response after adjustment for virological and histological confounders, which emphasizes the interplay between host and virological factors on the response to pegylated interferon/ribavirin therapy in Taiwanese CHC patients.

A number of factors have been considered as important predictors for the response to PEG-IFN/ribavirin therapy. Clinical parameters including non-genotype 1 infection, lower levels of

Table 5
Summary of significant factors associated with response to pegylated interferon-alpha 2a and ribavirin in group B (genotype 1b infection with viral load < or =5.6 log IU/ml or genotype non-1b infection) chronic hepatitis C patients.

	No.	Non-responder *n* (%), (*N* = 32)	Sustained responder *n* (%), (*N* = 135)	Univariate analysis			Multivariate analysis		
				OR	95% CI	*P*	OR	95% CI	*P*
Factor									
Hepatic fibrosis[a]		F: 3–4 = 1; F: 0–2 = 0		0.264	0.115–0.606	**0.001**	0.305	0.128–0.726	**0.007**
Alleles[b]									
A24	55	6 (18.8)	49 (36.3)	2.469	0.951–6.413	0.058	2.016	0.814–5.743	0.122
B40	68	18 (56.3)	50 (37.0)	0.458	0.210–0.999	**0.047**[#]	0.488	0.218–1.092	0.081
B46	36	11 (34.4)	25 (18.5)	0.434	0.186–1.014	0.058	0.407	0.168–0.988	**0.047**
DQB1*3	104	25 (78.1)	79 (58.5)	0.395	0.160–0.977	**0.040**[#]	0.406	0.160–1.027	0.057
DRB1*9	41	12 (37.5)	29 (21.5)	0.456	0.200–1.041	0.058	0.427	0.180–1.011	0.053
Haplotypes[c]									
B40-DRB1*3	6	3 (9.4)	3 (2.2)	0.220	0.942–1.144	0.085	0.179	0.032–0.989	**0.049**
B46- DRB1*9	19	7 (21.9)	12 (8.9)	0.348	0.125–0.973	**0.044**[#]	0.313	0.107–0.918	**0.034**
Cw1- DQB1*3	39	12 (37.5)	27 (20.0)	0.417	0.182–0.956	**0.035**[#]	0.350	0.145–0.845	**0.020**
Cw1- DRB1*9	24	9 (28.1)	15 (11.1)	0.319	0.125–0.817	**0.023**[#]	0.282	0.105–0.759	**0.012**
DQB1*3- DRB1*9	40	12 (37.5)	28 (20.7)	0.436	0.191–0.998	**0.046**[#]	0.412	0.174–0.978	**0.044**

Note: OR: odds ratio; CI: confidence interval.
[a] Multivariate analysis factors included were sex, age, pretreatment serum aspartate aminotransferase, alanine aminotransferase levels, and hepatic fibrosis.
[b] Multivariate analysis by hepatic fibrosis (allele possessing effect).
[c] Multivariate analysis by hepatic fibrosis.
[#] Bonferroni correction: not statistically significant.

viremia and the absence of cirrhosis have been currently reported to be associated with a better response (Hadziyannis et al., 2004). Our present study showed that pretreatment serum HCV RNA levels, HCV genotype and severity of liver fibrosis were also the main independent factors associated with SVR to PEG-IFN/ribavirin therapy in Taiwanese patients. We have reported that it is desirable to tailor and develop individualized treatment regimens according to the virological factors and degree of liver fibrosis for patients with CHC (Yu et al., 2004, 2006). With regard to the genetic background, it may be presumed that a potential immunological response could be significantly enhanced by PEG-IFN/ribavirin therapy in CHC patients. Effective presentation of viral antigens to $CD4^+$ T cells and $CD8^+$ T cells by HLA class II and class I molecules, respectively, is the key regulation of optimum immune response against viral infection. With the upregulated expression of immunogenetic molecules which enhances the immune response by IFN, the genetic variations at HLA loci with respect to antigen presentation might be a candidate related to response to IFN-based therapy. The B54, DR4, DRB1*07, and DRB1*13 alleles have been reported to be associated with non-response to IFN therapy, whereas the DRB1*0404, DRB1*0701, DQA1*0201, DQB1*02, and DR2 alleles were associated with SVR (Almarri et al., 1998; Alric et al., 1999; Sim et al., 1998; Thursz et al., 1999). In studying the relationship of HLA haplotypes and response to IFN therapy, HLA-B54 and HLA-A24-B54-DR4 haplotypes were reported as predictors for poor response (Kikuchi et al., 1998), and the DRB1*0701-DQA1*0201-DQB1*02 haplotype was associated with and response to IFN (Wawrzynowicz-Syczewska et al., 2000). We have also reported that in Taiwanese patients with CHC, the A11, B51, Cw15, and DRB1*15 alleles might be positively correlated with sustained response, whereas A24 might be associated with poor response to IFN therapy (Yu et al., 2003). These results suggest that HLA polymorphisms may play an important role in the response to IFN therapy in chronic HCV infection.

Jiao et al. have analyzed the frequency of HLA-DRB and their response to IFN-alpha and ribavirin in 25 Chinese patients indicating most female patients with HCV 2b and HLA-DRB1*07 presented complete response, whereas male patients with HCV 1b and HLA-DRB1*04 usually demonstrated no response (Jiao and Wang, 2005). Reports by Romero-Gómez et al. in 105 Spanish CHC patients (46 naïve and 59 relapsers) showed that HLA B44 is related to a higher rate of SVR in combination therapy with IFN and ribavirin but not in IFN monotherapy (Romero-Gómez et al., 2003). In the present study, the first report with the largest patient number, we found that no single HLA class II allele was associated with response to PEG-IFN/ribavirin therapy. Nevertheless the analyses of haplotypes showed that B40-DRB1*3, B46-DRB1*9, Cw1- DQB1*3, and Cw1- DRB1*9 might be associated with non-response. The study on associations between alleles and haplotypes and response to combination treatment have been verified by adjusting and controlling the important virological and histologic confounders (pretreatment serum HCV RNA levels, HCV genotype and liver fibrosis) by a logistic regression model. Our results did offer an evidence for the role of immunogenetics in the response to PEG-IFN/ribavirin therapy.

In Taiwan about one-sixth of the general population (Shaw et al., 1999) and one quarter to one third of CHC patients are indeed heterozygous or homozygous for A24 (Yu et al., 2003). Our previous study also showed that A24 inversely correlated with a response to IFN treatment, both qualitatively and quantitatively (Yu et al., 2003). The A24 frequency is around 10% in Caucasian population (Schipper et al., 1997) and 15–50% in Pacific/East Asians (Tokunaga et al., 2001). No association, between A24 and the therapeutic response to combination therapy with conventional IFN and ribavirin was demonstrated by Romero-Gómez et al. (2003). The addition of ribavirin might have influenced the association between A24 and the response to IFN therapy for CHC. It is noteworthy that in our data, homozygote-genotype analysis showed that A24 probably had a gene-dosage effect on the sustained response to PEG-IFN/ribavirin therapy. Kurokohchi et al. reported an immunodominant HLA-A24 restricted CTL epitope presented by HLA-A24 molecule was identified using a series of synthetic peptides containing the HLA-A24 binding motifs (Kurokohchi et al., 2001). Immunologic factors are important as recovery from acute viral hepatitis is associated with an unopposed TH1 response exposed to HCV and is also enhanced following treatment, associated with a SVR in chronic HCV infection (Bowen and Walker, 2005). Ribavirin, possessing activity against several RNA and DNA viruses, was suggested to have a mechanism of action to modulate the immune system by promoting T helper cell (T_H) 1 over T_H2 phenotype (Lau et al., 2002). Whether the addition of ribavirin can explain the opposite effects of A24 on the IFN monotherapy and PEG-IFN/ribavirin therapy needs further investigation.

Our previous study has shown that the HLA allele was associated with response in patients with HCV genotype 2 infection and low viral loads but not in other patients with HCV genotype 1b infection and/or high pretreatment viral loads (Yu et al., 2003). The DRB alleles have also been reported to be associated with response in Caucasian genotype 1b infection with IFN monotherapy (Alric et al., 1999) and in Chinese male patients with genotype 1b infection and female patients with genotype 2b infection with combination therapy with conventional IFN and ribavirin (Jiao and Wang, 2005). Our present study showed the influence of HLA alleles on the response to PEG-IFN/ribavirin therapy in patients with genotype 1b infection with viral load < or =5.6 log IU/ml or genotype non-1b infection but not in patients with genotype 1b infection and with a high viral load. Stratifying CHC patients by clinical variables, such as virological, histological and host immunogenetic factors, which are able to predict the therapeutic response is fundamental to develop the personalized therapy. This study supported the different importance of HLA among specific groups of patients stratified by virological determinants and the necessity in consideration of both virological and host genetic factors to develop individualized therapy for CHC patients.

In conclusion, our study suggested that not only virological but also host immunogenetic factors may possibly predict the response to PEG-IFN/ribavirin combination therapy in CHC patients. Further studies seem mandatory to confirm the possible associations between HLA alleles and HCV responses in an independent cohort. It will be also valuable to identify potential immunogenetic predictors which will potentially help selecting CHC patients suitable for combination therapy as well as designing individualized regimens for specific groups of CHC patients in clinical practice.

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